information, the LIF spectra are modulated by wavelength-dependent tissue attenuation.

The MAM Classifier

In accordance with the invention, a new mathematical classifier has been developed and applied to categorize spectral data for ischemia or hypoxia detection. The recent multicriteria associative memories (MAM) technique is modified to perform as a data classifier. Similar to existing classifiers, the MAM requires initial training on a set of input-output data pairs. The training process of the MAM classifier involves the calculation of the weights matrix $M(\eta)$ from the input-output data set using the following learning rule:

$$M(\eta)=\eta RS^T[\eta SS^T+(1-\eta)I]^{-1} \quad (17)$$

The matrices "S" and "R" holds the input and output training vectors as their columns, respectively. The superscripts ^{T} and $^{-1}$ indicate matrix transpose and inversion, respectively, and "I" is the identity matrix. The parameter η is initially set to 0.98; however, it can assume any value between 0 and 1 depending on the noise of the system. Following the training stage, the output "r" for an unknown input "s" can be readily calculated from the dot product:

$$r=M(\eta).s \quad (18)$$

Finally, an appropriate transfer function is used to assign the output "r" into one of several predetermined classification categories.

In the present invention, the MAM classifier is initially trained with LIFA (or absorbance) spectra acquired from normal or ischemic tissue as the training inputs. The corresponding normal or ischemic state can be encoded as, for example, "−1" or "1," respectively and used as the training outputs. Therefore, the training LIFA spectra are placed as columns of the input matrix "S" while their corresponding state-coded values are arranged in the same order as elements of the output row vector "R." The number of columns in "S" and elements in "R" are equal to the number of available training sets. A trained MAM matrix can determine whether an unknown LIFA spectrum "$s_?$" has been acquired from normal or ischemic tissue by calculating the dot product $r_?=M(\eta).\ s_?$ and passing the scalar result "$r_?$" to a hard limit transfer function. The hard limit transfer function then converts any negative or positive values of "$r_?$" into "0" or "1" indicating a normal or ischemic classification, respectively.

In a similar fashion, the MAM classifier can be applied for the detection of hypoxia and the discrimination between normal, ischemic and hypoxic tissue. It should be apparent that common or intrinsic LIF spectra can replace LIFA spectra as the classifier input. In addition, this input can be an entire spectrum, a re-sampled version of a spectrum or a set of statistical parameters or features characterizing a spectrum. Similarly, actual ischemia or hypoxia levels can be used as the classifier outputs instead of the binary coded output values employed in the above demonstration. In this case a linear transfer function may be used to categorize the output "r" of the MAM classifier.

The MAM technique outperformed the commonly-used artificial neural network (ANN) classifier in accurately classifying LIF/LIFA spectra resulting from normal and ischemic tissue. The superiority of the MAM classifier is most probably due to its insensitivity to spectral noise that might be present in LIF/LIFA spectra measured from biological systems. Although the foregoing MAM classifier is currently applied to discriminate spectral data for the purpose of ischemia or hypoxia detection, it is understood that those skilled in the art may apply it in various ways for different classification purposes.

Tissue Characterization

It will also be appreciated that the LIFAS devices and methods can be applied to tissue characterization, i.e., to differentiate between normal and diseased tissue, for tissue diagnostics and malignancy detection. Current LIFS techniques use the intensity spectrum of modulated LIF to identify malignant (cancerous and pre-cancerous) tissue and classify its type. LIFAS techniques offer a unique tissue characterizing capability not offered by conventional LIFS, based upon measurement of the attenuation spectrum.

A simple demonstration of LIFAS diagnostic capability is shown in FIGS. **17**(*a*)–(*d*). Although normal kidney and heart tissue are different in nature, their common LIF spectra, shown in FIGS. **17**(*a*) and (*b*) are almost identical and, hence, are not so useful for classification purposes. However, heart and kidney LIFA spectra, shown in FIGS. **17**(*c*) and *d* are different in terms of both shape and peak attenuation values. Thus, LIFAS techniques offer better tissue identification power than conventional LIFS techniques.

Other biomedical applications of the LIFAS methods and devices will include laser removal of decorative tattoos, detection of the in-vivo glucose level, assessment of the degree of burn trauma, detection of atherosclerotic plaque, angioplasty, measurement of acidity or alkalinity, pH measurement, the analysis of biochemical fluids, and the like. For example, measurement of the in vivo skin absorption using LIFAS methods and devices in accordance with the present invention can aid in the selection of optimal laser wavelengths for removing tattoos of different colors. Furthermore, since LIFAS techniques can be used to determine absorbance from a hypodermic sample volume, the skin color and the depth of the tattoo dye can be more accurately characterized than in surface reflectance techniques. Similarly, burn injury assessment can be accomplished by using LIFAS techniques to measure the depth of burn by probing for the presence of blood perfusion at varying locations within the tissue. LIFAS techniques can also be used to determine the absorbance or turbidity of a liquid in-situ without the necessity of extracting a sample for use in a spectrometer.

It will be understood by those of ordinary skill in the art that, although the LIFAS system and method is shown in the exemplary method as applied to biological tissue, it is also readily applicable to chemical and industrial material. For example, LIFAS devices and methods can be used to measure the absorbance and/or turbidity of materials and mixtures in medical, food, beverage, detergent, plastic, glass, oil, paint, textile, and semiconductor applications. Furthermore, the concentration of the pure components of the mixture can be determined from the absorbance spectrum using chemometric techniques such as multivariate regression (MLR), partial least squares (PLS) or artificial neural networks described above.

Although the foregoing discloses preferred embodiments of the present invention, it is understood that those skilled in the art may make various changes to the preferred embodiments without departing from the scope of the invention. The invention is defined only by the following claims.

We claim:

1. A spectroscopic method of analyzing a sample, comprising:

irradiating a sample with radiation to produce fluorescence from the sample, wherein the fluorescence is modulated by the sample;

monitoring a first portion of the modulated fluorescence at a first distance from the sample;

monitoring a second portion of the modulated fluorescence at a second distance from the sample, the second distance being different from the first distance;

comparing the first and second portions of the modulated fluorescence to determine a modulation characteristic of the sample.

2. The method of claim 1, wherein the radiation comprises substantially monochromatic light.

3. The method of claim 1, wherein the radiation comprises laser light.

4. The method of claim 1, wherein irradiating the sample comprises directing radiation at the sample using a waveguide.

5. The method of claim 4, wherein the waveguide is an optical fiber.

6. The method of claim 4, wherein the waveguide is an optical fiber bundle.

7. The method of claim 1, wherein monitoring of the modulated fluorescence comprises:

collecting a portion of the modulated fluorescence; and

determining the intensity of the collected portion of modulated fluorescence.

8. The method of claim 7, wherein the first portion of the modulated fluorescence is collected with a first waveguide and the second portion of the modulated fluorescence is collected with a second waveguide.

9. The method of claim 8, wherein the first waveguide is an optical fiber.

10. The method of claim 8, wherein the first waveguide is an optical fiber bundle.

11. The method of claim 8, wherein the second waveguide is an optical fiber.

12. The method of claim 8, wherein the second waveguide is an optical fiber bundle.

13. The method of claim 1, wherein irradiating the sample comprises directing radiation to the sample using a first waveguide and wherein the fluorescence is monitored using the first waveguide.

14. The method of claim 7, wherein the intensity of the collected portion of the fluorescence is determined with a sensor.

15. The method of claim 7, wherein the intensity of the first portion of the modulated fluorescence is determined with a sensor.

16. The method of claim 7, wherein the intensity of the second portion of the modulated fluorescence is determined with a sensor.

17. The method of claim 7, wherein the intensity of the first portion of the modulated fluorescence is determined with a first sensor and the intensity of the second portion of the modulated fluorescence is determined with a second sensor.

18. The method of claim 7, wherein the first and second portions of the modulated fluorescence are measured consecutively.

19. The method of claim 7, wherein the first and second portions of the modulated fluorescence are measured simultaneously.

20. The method of claim 11, wherein the method further includes determining the intrinsic fluorescence of the sample.

21. The method of claim 1, wherein the sample is biological material.

22. The method of claim 21, wherein the biological material is living tissue.

23. The method claim of 21, wherein the method further includes determining a physiological property of the biological material using the modulation characteristic.

24. The method of claim 21, wherein the method further includes determining a pathological property of the biological material using the modulation characteristic.

25. The method of claim 22, wherein the method further includes determining a physiological property of the living tissue using the modulation characteristic.

26. The method of claim 25, wherein the physiological property of the tissue is tissue oxygenation.

27. The method of claim 22, wherein the method further includes determining a pathological property of the tissue using the modulation characteristic.

28. The method of claim 27, wherein the pathological property of the tissue is the malignant condition of the tissue.

29. The method of claim 1, wherein either but not both of the distances is substantially zero.

30. A spectroscopic method of analyzing a sample, comprising:

irradiating a sample with radiation to produce return radiation from the sample, wherein the return radiation is modulated by the sample;

monitoring a first portion of the modulated return radiation at a first distance from the sample;

monitoring a second portion of the modulated return radiation at a second distance from the sample;

processing the first and second portions of the modulated return radiation to determine a modulation characteristic of the sample,

wherein the return radiation is modulated by attenuation.

31. The method of claims 30, wherein the return radiation is attenuated by scattering.

32. The method of claim 30, wherein the return radiation is attenuated by absorption.

33. The method of claim 30, wherein the modulation characteristic of the sample is attenuation.

34. The method of claim 30, wherein the modulation characteristic of the sample is absorption.

35. The method of claim 34, wherein the method further includes determining transmittance.

36. The method of claim 30, wherein the modulation characteristic of the sample is optical rotation.

37. A spectroscopic method of analyzing a sample, comprising:

irradiating a sample with radiation to produce return radiation from the sample, wherein the return radiation is modulated by the sample;

monitoring a first portion of the modulated return radiation at a first distance from the sample;

monitoring a second portion of the modulated return radiation at a second distance from the sample;

processing the first and second portions of the modulated return radiation to determine a modulation characteristic of the sample;

wherein the sample is biological material;

wherein the method further includes determining a physiological property of the tissue using the modulation characteristic; and

wherein the physiological property of the tissue is hypoxia.

38. A spectroscopic method for determining the oxygenation of a biological material, comprising:

irradiating a sample of a biological material with radiation to produce fluorescence from the sample, wherein the fluorescence is modulated by attenuation of the sample;

monitoring a first portion of the modulated fluorescence at a first distance from the sample;

monitoring a second portion of the modulated fluorescence at a second distance from the sample, the second distance being different from the first distance;

comparing the first and second portions of the modulated fluorescence to determine the attenuation of the sample;

determining oxygenation of the sample using the attenuation of the sample.

39. A spectroscopic method for determining the oxygenation of a biological material, comprising:

irradiating a sample of a biological material with radiation to produce return radiation from the sample, wherein the return radiation is modulated by attenuation of the sample;

monitoring a first portion of the modulated return radiation at a first distance from the sample;

monitoring a second portion of the modulated return radiation at a second distance from the sample;

processing the first and second portions of the modulated return radiation to determine the attenuation of the sample;

determining oxygenation of the sample using the attenuation of the sample;

wherein the oxygenation of the sample is determined by comparing the attenuation of the sample to the attenuation of a sample having a known level of oxygenation.

40. A spectroscopic method for determining the concentration of hemoglobin in a biological material, comprising:

irradiating a sample of biological material with radiation to produce fluorescence from the sample, wherein the fluorescence is modulated by attenuation of the sample;

monitoring a first portion of the modulated fluorescence at a first distance from the sample;

monitoring a second portion of the modulated fluorescence at a second distance from the sample, the second distance being different from the first distance;

comparing the first and second portions of the modulated fluorescence to determine the attenuation of the sample;

determining the concentration of hemoglobin in the sample using the attenuation of the sample.

41. A spectroscopic method for determining the concentration of hemoglobin in a biological material, comprising:

irradiating a sample of a biological material with radiation to produce return radiation from the sample, wherein the return radiation is modulated by attenuation of the sample;

monitoring a first portion of the modulated return radiation at a first distance from the sample;

monitoring a second portion of the modulated return radiation at a second distance from the sample;

determining the concentration hemoglobin in the sample using the attenuation of the sample;

wherein the concentration of hemoglobin is determined by comparing the attenuation of the sample to the attenuation of a sample having a known concentration of hemoglobin.

42. A method for determining a physiological characteristic of a biological material, comprising:

irradiating a sample of biological material with radiation to produce fluorescence from the sample, wherein the fluorescence is modulated by the sample;

monitoring a first portion of the modulated fluorescence at a first distance from the sample;

monitoring a second portion of the modulated fluorescence at a second distance from the sample, the second distance being different from the first distance;

comparing the first and second portions of the modulated fluorescence, using a predictive model, to determine a physiological characteristic of the sample.

43. A method for determining a physiological characteristic of a biological material, comprising:

irradiating a sample of a biological material with radiation to produce return radiation from the sample, wherein the return radiation is modulated by the sample;

monitoring a first portion of the modulated return radiation at a first distance from the sample;

monitoring a second portion of the modulated return radiation at a second distance from the sample;

processing the first and second portions of the modulated return radiation, using a predictive model, to determine a physiological characteristic of the sample;

wherein the predictive model is a multivariate linear regression.

44. A method for determining a physiological characteristic of biological material, comprising:

irradiating a sample of biological material with radiation to produce fluorescence from the sample, wherein the fluorescence is modulated by the sample;

monitoring a first portion of the modulated fluorescence at a first distance from the sample;

monitoring a second portion of the modulated fluorescence at a second distance from the sample, the second distance being different from the first distance;

comparing the first and second portions of the modulated fluorescence to determine a modulation characteristic of the sample;

processing the modulation characteristic using a predictive model to determine a physiological characteristic of the sample.

45. A method for determining a physiological characteristic of a biological material, comprising:

irradiating a sample of a biological material with radiation to produce return radiation from the sample, wherein the return radiation is modulated by the sample;

monitoring a first portion of the modulated return radiation at a first distance from the sample;

monitoring a second portion of the modulated return radiation at a second distance from the sample;

processing the first and second portions of the modulated return radiation, using a predictive model, to determine a physiological characteristic of the sample;

wherein the predictive model is a multicriteria associative memory classifier.

46. Apparatus for analyzing a sample, comprising:

a source adapted to emit radiation that is directed at a sample to produce fluorescence from the sample, wherein the fluorescence is modulated by the sample;

a first sensor adapted to monitor the fluorescence at a first distance from the sample and generate a first signal indicative of the intensity of the fluorescence;

a second sensor adapted to monitor the fluorescence at a second distance from the sample and generate a second signal indicative of the intensity of the fluorescence, the second distance being different from the first distance; and

a processor associated with the first sensor and the second sensor and adapted to compare the first and second signals to determine a modulation characteristic of the sample.

47. The apparatus of claim 46, wherein fiber optics transmit the fluorescence to the sensors.

48. Apparatus for analyzing a sample, comprising:

a source adapted to emit radiation that is directed at a sample volume in a sample to produce fluorescence

from the sample, such fluorescence including modulated fluorescence resulting from modulation by the sample;

a first sensor adapted to monitor the fluorescence at a first distance from the sample volume and generate a first signal indicative of the intensity of the fluorescence;

a second sensor adapted to monitor the fluorescence at a second distance from the sample volume and generate a second signal indicative of the intensity of the fluorescence, the second distance being different from the first distance;

a processor associated with the first sensor and the second sensor and adapted to compare the first and second signals to determine a modulation characteristic of the sample.

49. Apparatus for determining a modulation characteristic of a biological material, comprising:

a source adapted to emit excitation light;

a first waveguide disposed a first distance from the sample adapted to transmit the excitation light from the light source to the biological material to cause the biological material to produce fluorescence and adapted to collect a first portion of the fluorescence;

a first sensor, associated with the first waveguide, adapted to measure the intensity of the first portion of the fluorescence and generate a first signal indicative of the intensity of the first portion of the fluorescence;

a second waveguide disposed at a second distance from the sample adapted to collect a second portion of the fluorescence, the second distance being different from the first distance;

a second sensor, associated with the second waveguide, adapted to measure the intensity of the second portion of the fluorescence and generate a second signal indicative of the intensity of the second portion of the fluorescence;

a processor adapted to compare the first and second signals to determine a modulation characteristic of the biological material.

50. Apparatus for analyzing a sample, comprising:

a source adapted to emit radiation that is directed at a sample volume in a sample to produce fluorescence from the sample, such fluorescence including modulated fluorescence resulting from modulation by the sample;

a first sensor, displaced by a first distance from the sample volume adapted to monitor the fluorescence and generate a first signal indicative of the intensity of the fluorescence; and

a second sensor, displaced by a second distance from the sample volume adapted to monitor the fluorescence and generate a second signal indicative of the intensity of fluorescence, the second distance being different from the first distance;

a processor associated with the first sensor and the second sensor and adapted to compare the first and second signals to determine a physiological property of the sample.

51. Apparatus for determining a physiological property of biological material, comprising:

a source adapted to emit excitation light;

a first waveguide disposed a first distance from the sample adapted to transmit the excitation light from the light source to the biological material to cause the biological material to produce fluorescence and adapted to collect a first portion of the fluorescence;

a first sensor, associated with the first waveguide, for measuring the intensity of the first portion of the fluorescence and generating a first signal representative of the intensity of the first portion;

a second waveguide disposed at a second distance from the sample adapted to collect a second portion of the fluorescence, the second distance being different from the first distance;

a second sensor, associated with the first waveguide, for measuring the intensity of the second portion of the fluorescence and generating a second signal representative of the intensity of the second portion;

a processor adapted to compare the first and second signals to determine a physiological property of the biological material.

52. A spectroscopic method of analyzing a sample, comprising:

irradiating a sample with radiation to produce fluorescence from the sample, wherein the fluorescence is modulated by the sample;

monitoring a first portion of the modulated fluorescence at a first distance from the sample;

monitoring a second portion of the modulated fluorescence at a second distance from the sample, the second distance being different from the first distance;

comparing the first and second portions of the modulated fluorescence to determine a modulation characteristic of the sample;

wherein the sample is biological material;

wherein the method further includes determining a physiological property of the tissue using the modulation characteristic; and

wherein the physiological property of the tissue is ischemia.

53. A method for determining a physiological characteristic of a biological material, comprising:

irradiating a sample of a biological material with radiation to produce fluorescence from the sample, wherein the fluorescence is modulated by the sample;

monitoring a first portion of the modulated fluorescence at a first distance from the sample;

monitoring a second portion of the modulated fluorescence at a second distance from the sample, the second distance being different from the first distance;

comparing the first and second portions of the modulated fluorescence, using a predictive model, to determine a physiological characteristic of the sample;

wherein the predictive model is multivariate.

54. A spectroscopic method of analyzing a sample, comprising:

irradiating a sample with radiation to produce fluorescence from the sample, wherein the fluorescence is modulated by the sample;

monitoring a first portion of the modulated fluorescence at a first angle from the sample

monitoring a second portion of the modulated fluorescence at a second angle from the sample

comparing the first and second portions of the modulated fluorescence to determine a modulation characteristic of the sample.

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